บทความวิจัย

การกระจายตัวของเซลล์อิมมูโนรีแอกทีฟ Bcl-2 ในสมองของปลาซิวข้าวสาร *(Oryzias minutillus,* Teleostei*)*

อรินทม์ งามนิยม[1*], บุษบา ปันยารชุน[2], รักชนก โคโต[3] และ สุภาพร สุกสีเหลือง[1]

บทคัดย่อ

ในการวิจัยนี้เพื่อทำการศึกษาและเก็บข้อมูลตำแหน่งการกระจายตัวของโปรตีน Bcl-2 ในสมองของปลาซิวข้าวสาร *(Oryzias minutillus)* โดยการย้อมเนื้อเยื่อทางอิมมูโนฮิสโตเคมิสทรี จากผลการทดลองไม่สามารถตรวจพบเซลล์อิมมูโนรีแอกทีฟ Bcl-2 ที่สมองส่วนหน้าบริเวณเทเลนเซฟาลอน แต่สามารถตรวจพบเซลล์อิมมูโนรีแอกทีฟนี้ได้ที่ส่วนไดเอนเซฟาลอนแต่มีการแสดงออกของโปรตีนนี้ค่อนข้างน้อย เซลล์อิมมูโนรีแอกทีฟ Bcl-2 จะพบกระจายอยู่ทั่วในส่วนของสมองส่วนกลาง โดยพบโปรตีน Bcl-2 จำนวนมากที่บริเวณ proximal pars distalis และ pars nervosa ของ hypophysis ในส่วนของสมองส่วนหลัง การกระจายตัวของโปรตีน Bcl-2 สามารถพบได้ง่ายทั้งบริเวณมีเทนเซฟาลอนและไมอีเลนเซฟาลอน อย่างไรก็ตามเมื่อเปรียบเทียบการกระจายตัวของโปรตีนนี้ระหว่างปลาซิวเพศผู้และเพศเมียพบว่ามีลักษณะการกระจายตัวคล้ายกัน จากการศึกษานี้สรุปได้ว่า โปรตีน Bcl-2 มีการกระจายตัวอย่างเฉพาะที่ในแต่ละบริเวณของสมองทั้งสามส่วน แต่การกระจายตัวจะไม่มีความแตกต่างระหว่างเพศของปลาชนิดนี้ สำหรับการศึกษาครั้งนี้เป็นรายงานครั้งแรกถึงการกระจายตัวของโปรตีน Bcl-2 ในสมองของปลาซิวข้าวสาร ซึ่งผลการทดลองที่ได้นี้ช่วยเพิ่มข้อมูลและความก้าวหน้าในการศึกษากลไกต่างๆ ของโปรตีน Bcl-2 ในปลาจีนัสนี้ซึ่งนิยมใช้เป็นสัตว์ทดลองต้นแบบต่อไป

คำสำคัญ: Bcl-2 เซลล์อิมมูโนรีแอกทีฟ สมอง ปลาซิวข้าวสาร

[1] สถาบันพัฒนาการท่องเที่ยวเพื่ออนุรักษ์สิ่งแวดล้อม มหาวิทยาลัยศรีนครินทรวิโรฒ

[2] ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ

[3] ภาควิชาชีววิทยา มหาวิทยาลัยศรีนครินทรวิโรฒ

[*] ผู้นิพนธ์ประสานงาน, e-mail: arin16@hotmail.com

Distribution of Bcl-2 Immunoreactive Cells in Brain of Thai Medaka, *Oryzias minutillus* (Teleostei)

Arin Ngamniyom[1], Busaba Panyarachun[2], Rakchanok Koto[3], Supaporn Suksileung[1]

ABSTRACT

In this study, we examined the histological distribution of Bcl-2 protein in the fish brain of Thai medaka, *Oryzias minutillus,* by means of immunostaining procedures. In forebrains, no Bcl-2 immunoreactive (IR) cells were stained in the part of telencephalon. In contrast, IR cells were found along the part of diencephalon, but the expressions were weak. In midbrains, IR cells were widely distributed throughout these portions. Moreover, in the hypophysis, IR cells were strongly detected in the areas of proximal pars distalis and the pars nervosa. In hindbrains, immunoreactive localizations of Bcl-2 were mostly found in the both of metencephalon and myelencephalon parts. Distribution and localization of Bcl-2 IR cells were not different between males and females of Thai medaka. Our results suggest that the distribution of Bcl-2 protein may be region-specific expressions in Thai medaka brain but not sexual dimorphism. It is the first report of Bcl-2 distribution protein in Thai medaka. We support that immunohistological analysis can supply important data regarding the Bcl-2 distribution profile of fish brain and can be able to contribute to systematic studies as animal model of teleost.

Keywords: Bcl-2, immunoreactive cells, brain, Thai medaka

[1]Institute of Ecotourism, Srinakharinwirot University

[2]Department of Anatomy, Faculty of Medicine, Srinakharinwirot University

[3]Department of Biology, Faculty of Science, Srinakharinwirot University

Introduction

In many vertebrates, B-cell lymphoma (Bcl) 2 is a member of Bcl-2 family that plays central roles in the regulation of apoptotic pathways in several tissues [1-3]. In teleost fish, the Bcl-2 is critical mediators of the delicate balance between survival and apoptosis [4] and is expressed in liver, ovary, testis and lymphoid organ of Atlantic cod, *Gadus morhua,* piau-jejo, *Leporinus taeniatus,* gudgeon, *Gobio gobio* and zebrafish, *Danio rerio,* respectively [5-8]. Kratz et al. [9] also reported that the expressions of Bcl-2 family were found in the brain of zebrafish. However, the data profiling of Bcl-2 in the teleost fish remain to be elucidated.

It is known that Thai medaka, *Oryzias minutillus,* is a relative genus of Japanese medaka (*Oryzias latipes,* Teleostei) which is one of the best model organisms for experimental vertebrate in various fields [10] such as cell biology [11], developmental biology [12] and neurobiology [13]. This species, which is the smallest species among genus *Oryzias,* is widely distributed in Thailand [14]. The body size of Thai medaka is about the half of Japanese medaka [15]. Attributes of Thai medaka that should be encouraged its laboratory use include small size and ease of maintenance in fresh water aquarium [10, 16].

As the aim of this study, we provided an anatomical distribution and cellular basis of Bcl-2 immunoreactive cells in the brain of Thai medaka, *Oryzias minutillus.* Furthermore, we believed that elucidation of the Bcl-2 distribution profile in the brain contribute to increasing our knowledge of the Bcl-2 regulation in teleost fish.

Materials and methods

Fish

Adult Thai medaka of which standard length was 12-14 mm were captured in ponds in suburbs of Bangkok, Thailand, from April to May 2010. This period was the non-breeding season for Thai medaka [16]. Ten males or ten females were kept separately in aquaria with a controlled 12: 12 hr light/dark photoperiod cycle at 26°C for 2 weeks, and fed ad libitum with TetraMin (Tokyo, Japan). Their sexes were judged from the morphology of the secondary sex characters of the dorsal and anal fins, according to the criteria of Ngamniyom et al. [15].

Immunohistochemistry of Bcl-2

Brains of male, female fish were dissected out from the bodies and fixed in Bouin's solution without acetic acid for 12h, and stored in 70% ethanol. Brain fish was precisely separated to three parts, according to the criteria of Ishikawa et al. [17]. The part of tepencephalon to the part of diencephalon was distinguished from midbrain as the forebrain. The part of nervus opticus until the posterior end of tectum opticum was indentified such the midbrain. The

hindbrain composed of the metencephalon and myelencephalon. Brain sections were described in Fig. 1A and B. Paraffin sections of brain were prepared at 6 μm.

The primary antibody: anti-Bcl-2 (rabbit anti-Bcl-2 monoclonal antibody; Santa Cruz biotechnology, Inc., OR., USA) was diluted 1: 10,000 (final peptide concentration, 0.02 μg/ml). Those sections were incubated with the primary antibody for 16h, with the secondary anti-rabbit antibody (Dako, Glostrup, Denmark) for 30 min, with streptavidin (Dako, Glostrup, Denmark) for 30 min, and finally colorized with diaminobenzidine solution (Dojin Co. Ltd. Japan). Those sections were stained with hematoxylin as a counter staining. Immunoreactive cells, which gave brownish color in the brain, were observed under a light microscope (BX51 Olympus, USA).

The unpaired Student *t*-test was used to examine differences statistically.

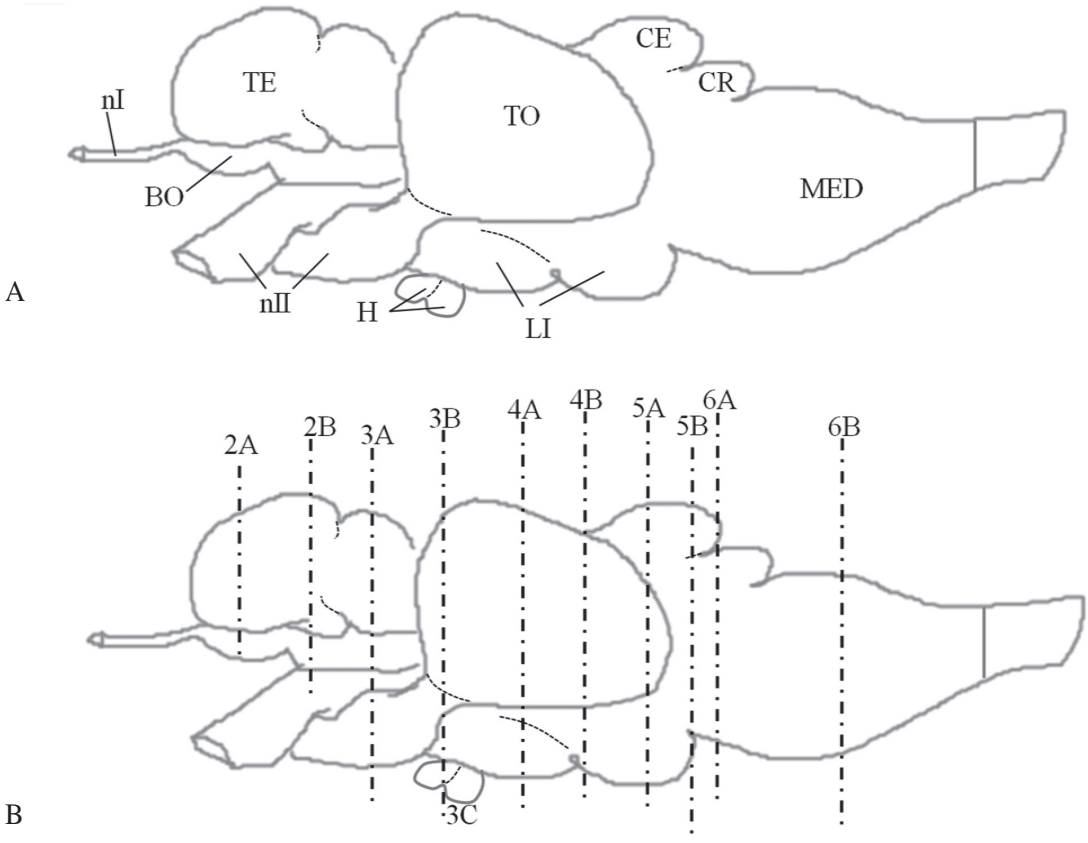

Fig. 1 Diagrammatic illustration of brain sections of Thai medaka (1A) and brain sections of each part (1B). The numbers with alphabet letters in Fig. 1B were related to Fig. 2, 3, 4, 5 and 6.

Nomenclature

We followed the neuroanatomical terms of Ishikawa et al. [17]. Other references were listed in the index of abbreviations (Table 1).

Results

It was noted that in this study, distribution and localization of Bcl-2 immunoreactive (IR) cells were not different between males and females of Thai medaka (unpaired Student *t*-test, $P > 0.05$) (Table 1).

Table 1 Distribution of Bcl-2 protein in brains of thai medaka

Forebrain			
Structure (abbreviation)	**Bcl-2 immunoreactive (IR) cell**	**Number of Bcl-2 IR cell**	
		Male (mean ± SE)	**Female (mean ± SE)**
bulbus olfactorius (BO)	-	-	-
area dorsalis telencephali pars lateralis (DI)	-	-	-
area dorsalis telencephali pars medialis (Dm)	-	-	-
dorsal region of Dm	-	-	-
area dorsalis telencephali pars posterior (Dp)	-	-	-
lateral forebrain bundle (lfb)	-	-	-
nucleus preopticus pars magnocellularis	+	6.6 ± 0.7	5.7 ± 0.5
(Pom) [32]			
nucleus preopticus pars parvocellularis	+	8.3 ± 0.6	8.9 ± 0.4
(Pop) [32]			
ventral region of Dm	-	-	-
area ventralis telencephali pars dorsalis (Vd)	-	-	-
area ventralis telencephali pars posterior (Vp)	+	7.3 ± 0.4	8.1 ± 0.4
area ventralis telencephali pars ventralis (Vv)	+	12.3 ± 0.6	9.8 ± 0.8
Midbrain			
commissura ansulata (cans)	-	-	-
commissura horizontalis (ch)	-	-	-
fasciculus longitudinalis medialis (flm)	+	4.3 ± 0.2	5.4 ± 0.3
granule population (G) [31]	+	7.5 ± 0.6	9.3 ± 0.4
corpus glomerulosum pars rotunda (GR)	+	3.5 ± 0.3	4.2 ± 0.6
hypophysis (H)	+	22.5 ± 0.9	25.3 ± 0.8

Table 1 (continue)

Forebrain			
Structure (abbreviation)	**Bcl-2 immunoreactive (IR) cell**	**Number of Bcl-2 IR cell**	
		Male (mean ± SE)	**Female (mean ± SE)**
nucleus anterior tuberis (NAT)	+	5.1 ± 0.2	6..3 ± 0.4
nucleus diffuses lobi inferioris (NDLI)	-	-	-
nucleus gustatorius secundarius (NGS)	+	6.4 ± 0.6	7.1 ± 0.7
nucleus interpeduncularis (NIP)	-	-	-
nucleus nervi trochlearis (NIV)	+	4.0 ± 0.5	5.3 ± 0.4
nucleus recessus lateralis (NRL) [32]	-	-	-
nucleus recessus postedoris (NRP) [32]	-	-	-
nucleus raphes (NRHP)	+	18.3 ± 0.5	23.4 ± 1.0
nucleus posterioris periventricularis NPPv) [32]	+	7.0 ± 0.7	11.1 ± 0.8
nucleus ventralis tuberi (NVT) [33]	-	-	-
nucleus preglomerulosus pars medialis (PGm)	-	-	-
medial reticular zone (RFm)	+	31.6 ± 1.7	35.3 ± 1.6
torus longitudinalis (TL)	-	-	-
tectum opticum (TO)	-	-	-
torus semicircularis (TS)	-	-	-
tractus mesencephalocerebellaris (tmc)	-	-	-
tractus tectobulbaris (ttbc)	-	-	-
tractus tectobulbaris cruciatus (ttbc)	-	-	-
valvula cerebella (VC)	+	3.2 ± 0.3	4.7 ± 0.6
ventriculus mesencephali (vem)	+	5.4 ± 0.6	4.3 ± 0.4
nucleus ventromedialis thalami (VM)	+	6.6 ± 1.1	12.1 ± 1.6
lemniscus lateralis (ll)	+	4.4 ± 0.4	3.7 ± 1.0
nervus oculomotorius (nlll)	-	-	-
Sulcus limitans telencephali [33]	-	-	-
Hindbrain			
cellula Mauthneri (M)	+	10.2 ± 0.9	11.9 ± 1.1
commissura ansulata (cans)	-	-	-
eminentia granularis (EG)	-	-	-
fasciculus longitudinalis medialis (flm)	+	6.1 ± 0.6	7.3 ± 0.5
magnocellular octavus nucleus (MCN)	+	7.8 ± 0.3	9.3 ± 0.7

Table 1 (continue)

Forebrain			
Structure (abbreviation)	Bcl-2 immunoreactive (IR) cell	Number of Bcl-2 IR cell	
		Male (mean ± SE)	Female (mean ± SE)
nervus lineae lateralis anterior (nALL)	+	11.0 ± 0.4	7.9 ± 1.2
nucleus raphes (NRPH)	-	-	-
nervus octavus (nVlll)	-	-	-
nucleus motorius nervi vagi (NXm)	-	-	-
medial reticular zone (RFm)	-	-	-
tractus tectobulbaris cruciatus (ttbc)	-	-	-
tractus tectobulbaris rectus (ttbr)	-	-	-
secondary octaval population (SO) [31]	+	14.8 ± 0.8	13.7 ± 0.7
lobus vagi (XL)	+	19.6 ± 1.4	17.7 ± 0.9
radix descendens nervi trigemini (tv)	+	4.6 ± 0.5	3.6 ± 0.6
tractus vestibulosplnalis (tvs)	+	3.7 ± 0.2	4.2 ± 0.5
nucleus motorius nervi vagi (NXm)	+	5.1 ± 0.6	4.2. ± 0.4

Note: The detection of Bcl-2 immunoreactive cells was +, and no detection was -. Male or female group consisted of ten samples of fish brains.

Bcl-2 immunoreactive cells in the forebrain of Thai medaka

No distribution of Bcl-2 IR cells was observed in any area of telencephalon (Table 1, Fig. 2A). In diencephalon part, Bcl-2 IR cells were found in area ventralis telencephali pars dorsalis (Vd) and area ventralis telencephali pars ventralis (Vv) (Fig. 2B). The staining intensity was numerous in area of nucleus preopticus pars parvocellularis (Pop) but was weak near the area of nervus opticus (nll) (Fig. 2B and 3A). Bcl-2 IR cells were also detected in area ventralis telencephali pars posterior (VP) and area of preopticus pars magnocellularis (Pom) (Fig. 3A).

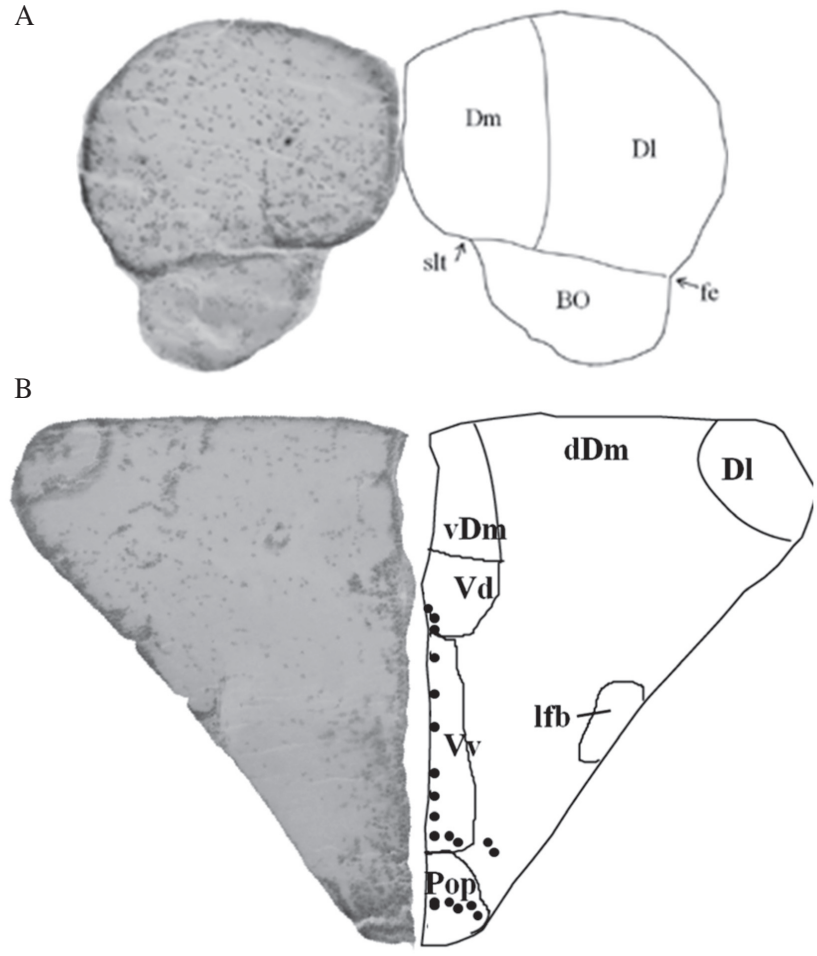

Fig. 2 Distribution of Bcl-2 immunoreactive cells in forebrains of Thai medaka. Rostal part (2A) and caudal part (2B). Bars = 200 μm

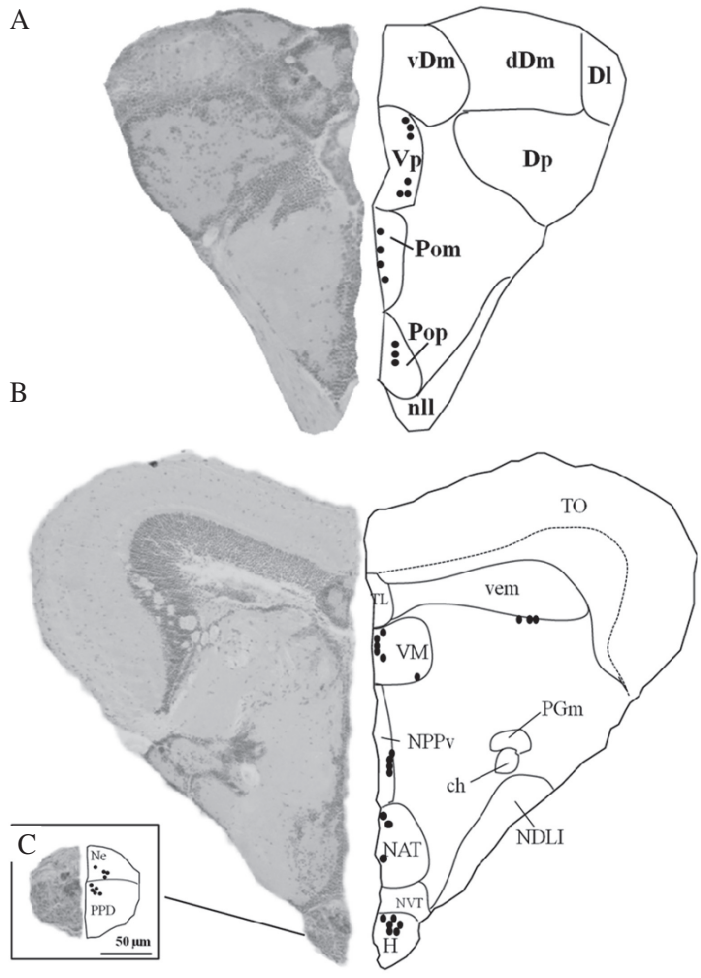

Fig. 3 Distribution of Bcl-2 immunoreactive cells in caudal end of forebrain (3A), rostral part of midbrain (3B) and hypophysis (3C). Bars = 200 µm.

Bcl-2 immunoreactive cells in the midbrain of Thai medaka

Bcl-2 IR cells were found along midbrain part (Table 1). Immunoreactivities of Bcl-2 were localized in areas of nucleus posterioris periventricularis (NPPV), nucleus anterior tuberis (NAT) (Fig. 3B) and valvula cerebelli (VC) (Fig. 4B). In hypophysis, Bcl-2 IR cells were observed intensely in proximal pars distalis (PPD) and the pars nervosa (Ne) (Fig. 3C). Localization of immunoreactive cells was found near the area of ventriculus mesencephali (Vem) (Fig. 3B). Bcl-2 IR cells were surrounded the areas of corpus glomerulosum pas rotunda (GR) and lemniscus lateralis (ll) (Fig. 4A and B). In the end part of midbrain, Bcl-2 IR cells were localized at fasciculus longitudinalis medialis (flm), granule population (G), nucleus gustatoris secundarius (NGS), medial recticular zone (RFm) and nucleus raphes (NRPH) (Fig. 4B and 5A).

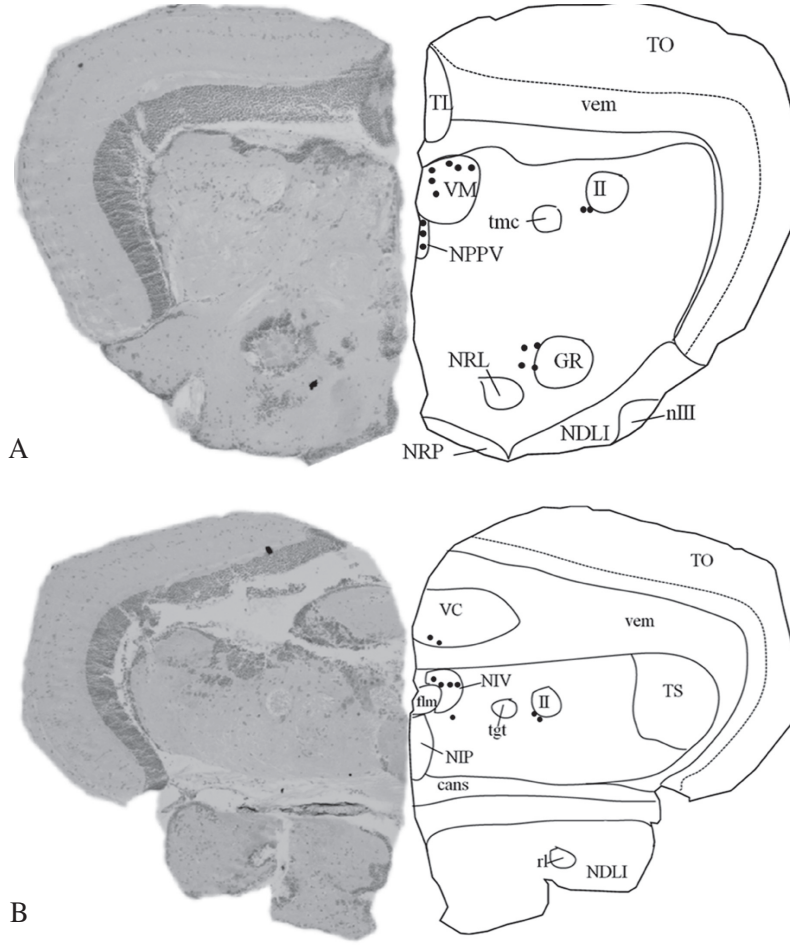

Fig. 4 Distribution of Bcl-2 immunoreactive cells in midbrains. Middle part (4A) and caudal part of midbrain (4B) Bars = 200 μm.

Bcl-2 immunoreactive cells in the hindbrain of Thai medaka

Bcl-2 IR cells were observed throughout hindbrain part (Table 1). Immunoreactivities of Bcl-2 were mostly found in corpus cerebelli (CE) and RFm (Fig. 5B, 6A and B). In cellula Mauthneri (M), there was the cluster of Bcl-2 IR cells (Fig. 5B). Cells with a thick and thin apical process were both immunoreactive in areas of RFm, M (Fig. 5B and 6A) and flm (Fig. 6B). However, it was hard to detect Bcl-2 IR cells in fasciculus longitudinalis medialis (Fig. 5B, 6A and B). IR cells were abundant in the nucleus medialis (MN) and secondary octaval population (SO) (Fig. 5B and 6A). Positive staining cells were also detected widely in magnocellular octavus nucleus (MCN) and nervus lineae lateralis anterior (nALL) (Fig. 5B, 6A). They were also present in high numbers inside area of lobus vagi (XL) and ventral edge of nucleus motorius nervi vagi (NXm) (Fig. 6B). In radix decendens nervi trigemini (tV) and tractus vestibulosplnalis (tvs), the weak IR cells were found in dorsal edges (Fig. 6B).

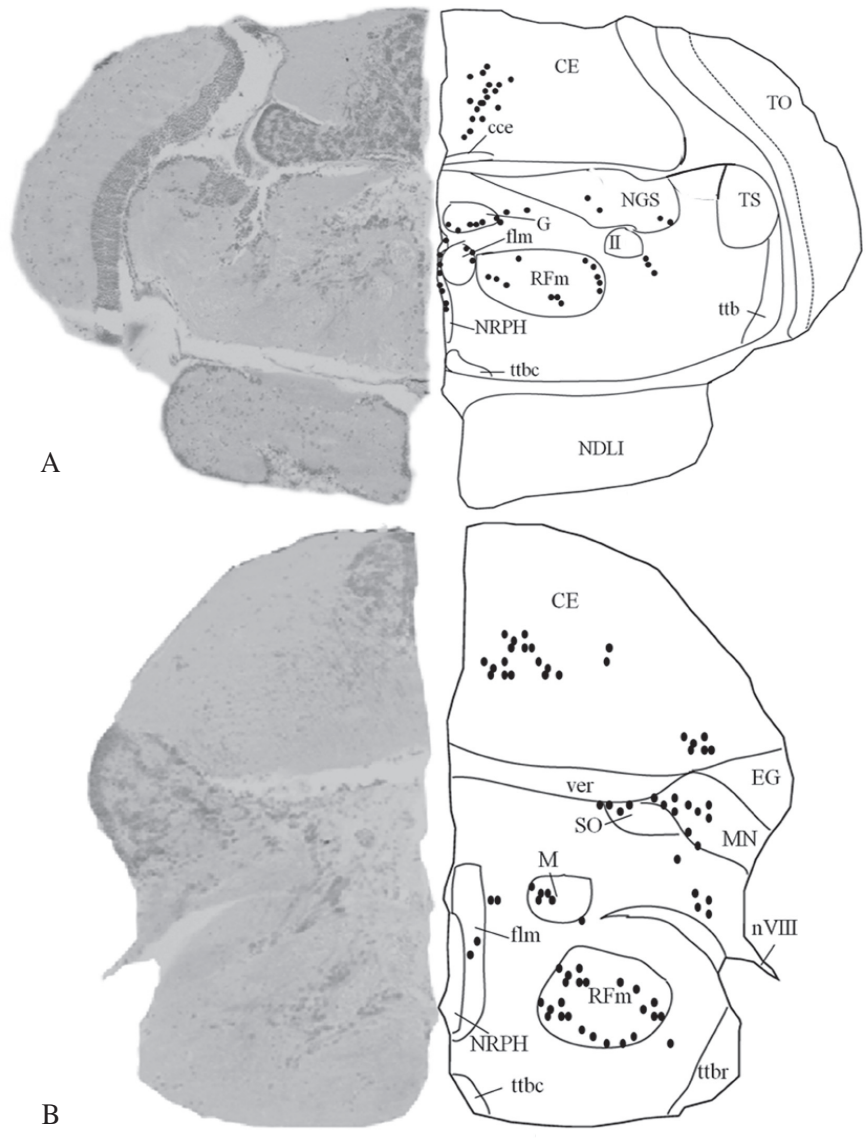

Fig. 5 Distribution of Bcl-2 immunoreactive cells in caudal end of midbrain (5A) and rostral part of hindbrain (5B). Bars = 200 μm.

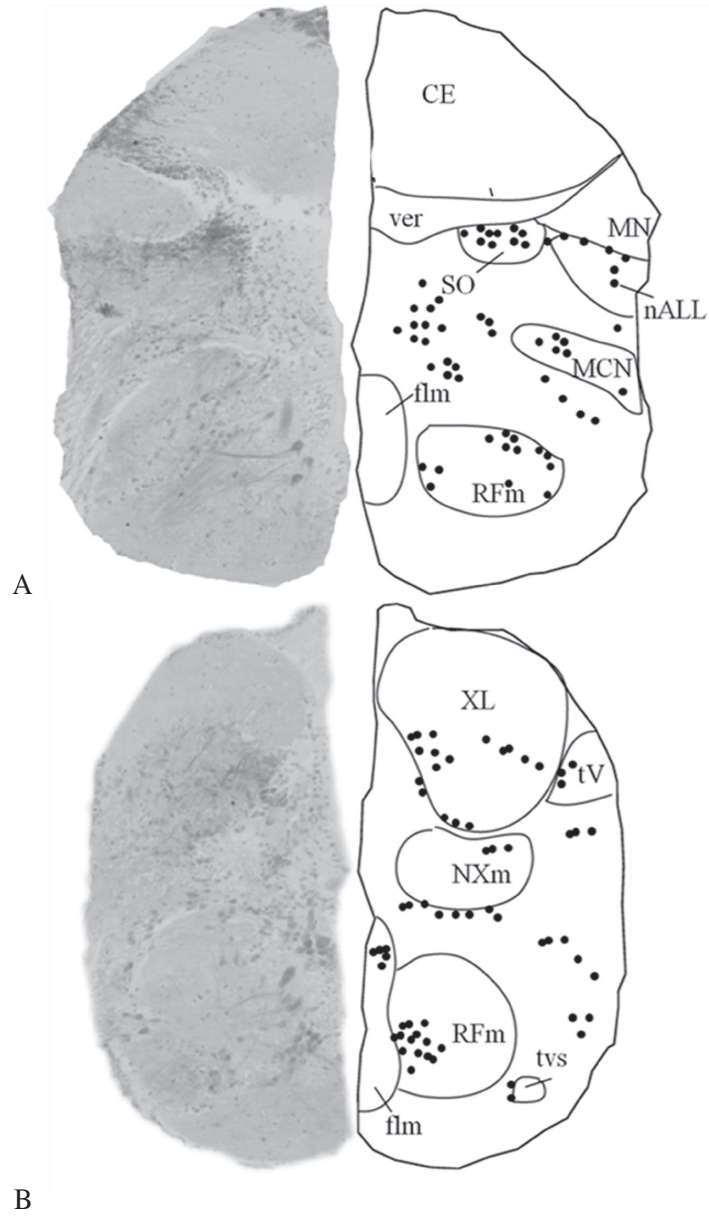

Fig. 6 Distribution of Bcl-2 immunoreactive cells in hindbrains. Middle part (6A) and caudal part of hindbrain (6B). Bars = 200 μm.

Discussion

In this study, we tried to clarify the distribution profile of Bcl-2 protein in fish by means of immunohistochemical procedures and found that the Bcl-2 protein distribution was brain region-specific. This finding was similar to previous reports of Vyas et al. [18], Zhang et al. [19], Lutz and Prentice [20] and Sick et al. [21] that the expressions of Bcl-2 protein were different in each brain region of human, rat and freshwater turtle (*Trachemys scripta*). Bcl-2 protein may be evolutionarily conserved distribution feature of region-specific in the brain of non-mammalian vertebrates to mammals.

Tsukahara et al. [22] and Forger et al. [23] reported that Bcl-2 protein levels in brains were different between males and females. In Bcl-2 protein distribution, however, there was not sex-specific between male and female brains. Therefore, Bcl-2 may exhibit sexual dimorphisms only in the expression levels in brain.

The distribution of Bcl-2 was examined in forebrain of mammal species [24, 25]. Mooney and Miller [26] reported that the expression of Bcl-2 protein was detected along diencephalon of rats. Shindler et al. [27] also reported that Bcl-2 was expressed in telencephalic cell cultures for playing a supportive role to Bcl-xL in maintaining telencephalic cell survival. In contrast, sections of telencephalon from Thai medaka did not exhibit any of immunoreactivity of Bcl-2 in our present study. It is known that Bcl-2 suppresses apoptotic pathways [9]. Therefore, expression of Bcl-2 protein may be weak or unnecessary for regulating an antiapoptotic role in telencephalic areas of Thai medaka.

In male and female Thai medaka, abundant positive cells of Bcl-2 were detected in edge midbrain near sulcus limitans. Bcl-2 positive cells were strongly expressed in pituitary gland of those fish. These findings are consistent with a report of Shin *et al.* [28] that density of Bcl-2 immunoreactive cells was high in midbrain portions of mice. In contrast, it was hard to detected positive immunostaining for Bcl-2 in normal pituitary of mammals [29, 30].

Similarly to midbrain of Thai medaka, Bcl-2 was highly expressed in hindbrain portion including a part of spinal cords. Shin et al. [28] also reported that Bcl-2 density was strongly detected in hindbrain and spinal cords of rats. It suggests that Bcl-2 may be necessary to some antiapoptotic pathways, regulating on survival cells of midbrain, pituitary gland, hindbrain and spinal cords in medaka fish.

Thus, the dwarf medaka may be provided a convenient model for studying Bcl-2 regulation involved in apoptosis because it has uncomplicated tissues, and most of the same organs are found in mammal species.

In summary, the present study is the first to demonstrate that the Bcl-2 protein distributed specifically in both male and female brains of a fresh-water teleost, although a

comprehensive understanding of regulation mechanism and physiological context remain to be elucidated.

References

1. Allsopp, T. E, Kiselev, S., Wyatt, S., and Davies, A. M. 1995. Role of Bcl-2 in the Brain-Derived Neurotrophic Factor Survival Response. *European Journal of Neuroscience* 7: 1266-1272.
2. Adams, J. M., and Cory, S. 1998. The Bcl-2 Protein Family: Arbiters of Cell Survival. *Science* 281: 1322-1326.
3. Germain, M., and Shore, G. C. 2003. Cellular Distribution of Bcl-2 Family Proteins. *Science Signaling* 2003: 1-10.
4. Hughes, P., Bouillet, P., and Strasser, A. 2006. Role of Bim and Other Bcl-2 Family Members in Autoimmune and Degenerative Diseases. *Current Directions in Autoimmunity* 9: 74-94.
5. Feng, C. Y., and Rise, M. L. 2010. Characterization and Expression Analyses of Anti-Apoptotic Bcl-2-like Genes NR-13, Mcl-1, Bcl-X1, and Bcl-X2 in Atlantic Cod (*Gadus morhua*). *Molecular Immunology* 47: 763-784.
6. Santos, H. B., Rizzo, E., Bazzoli, N., Sato, Y., and Moro, L. 2005. Ovarian Regression and Apoptosis in the South American Teleost *Leporinus taeniatus* Lutken (Characiformes, Anostomidae) from the Sao Francisco Basin. *Journal of Fish Biology* 67: 1446-1459.
7. Nadzialek, S., Pigneur, L. M., Wéron, B., and Kestemont, P. 2010. Bcl-2 and Caspase 3 mRNA Levels in the Testes of Gudgeon, Gobio gobio, Exposed to Ethinylestradiol (EE2). *Aquatic Toxicology* 98: 304-310.
8. Langenau, D. M., Jette, C., Berghmans, S., Palomero, T., Kanki, J. P., Kutok, J. L., and Look, A. T. 2005. Suppression of Apoptosis by Bcl-2 Overexpression in Lymphoid Cells of Transgenic Zebrafish. *Blood* 105: 3278-3285.
9. Kratz, E., Eimon, P. M., Mukhyala, K., Stern, H., Zha, J., Strasser, A., Hart, R., and Ashkenazi, A. 2006. Functional Characterization of the Bcl-2 Gene Family in the Zebrafish. *Cell Death and Differentiation* 13: 1631-1640.
10. Lynne, R. P. 2008. A Phylogenetic Analysis and Taxonomic Revision of Ricefishes, *Oryzias* and Relatives (Beloniformes, Adrianichthyidae). *Zoological Journal of the Linnean Society* 154: 494-610.
11. Hong, Y., and Schartl, M. 2006. Isolation and Differentiation of Medaka Embryonic Stem Cells. *Methods in Molecular Biology* 329: 3-16.
12. Karim, M., Danièle, P., Arul, M., Simone, P. D., and Marc, E. 2009. Medaka Proteome

Study: Use of Cross-Species Matching and Expressed Sequence Tag Data for Protein Identification. *Journal of Proteomics and Bioinformatics* 2: 67-77.

13. Alunni, A., Hermel, J. M., Heuzé, A., Bourrat, F., Jamen, F., and Joly, J. S. 2010. Evidence for Neural Stem Cells in the Medaka Optic Tectum Proliferation Zones. *Developmental Neurosciences* 70: 693-713.
14. Magtoon, W., and Uwa, H. 1985. Karyotype Evolution and Relationship of a Small Ricefish *(Oryzias minutillus)* from Thailand. *Proceedings of the Japan Academy* 61: 157-160.
15. Ngamniyom, A., Magtoon, W., Nagahama, Y., and Sasayama, Y. 2007. A Study of the Sex Ratio and Fin Morphometry of the Thai medaka (*Oryzias minutillus*) Inhabiting Suburbs of Bangkok, Thailand. *Fish Biology Journal of Medaka* 11: 17-21.
16. Ngamniyom, A., Magtoon, W., Nagahama, Y., and Sasayama, Y. 2009. Expression Levels of Hormone Receptors and Bone Morphogenic Protein in Fins of Medaka. *Zoological Science* 26: 74-79.
17. Ishikawa, Y., Yoshimoto, M., and Ito, H. 1997. A Brain Atlas of a Wild-Type Inbred Strain of the Medaka, *Oryzias latipes. Fish Biolology Journal of Medaka* 10: 1-26.
18. Vyas, S., Javoy-Agid, F., Herrero, M. T., Strada, O., Boissiere, F., Hibner, U., and Agid, Y. 2002. Expression of Bcl-2 in Adult Human Brain Regions with Special Reference to Neurodegenerative Disorders. *Journal of Neurochemistry* 69: 223-231.
19. Zhang, H. X., Li, X. Q., and Tang, T. 2003. Characteristics of Bcl-2 and Bax Expressions in Rat Brain After Experimental Intracerebral Hemorrhage and Intervention of Naoyian Granule. *Hunan Yi Ke Da Xue Xue Bao* 28: 229-232.
20. Lutz, P. L., and Prentice, H. 2002. Sensing and Responding to Hypoxia, Molecular and Physiological Mechanisms. *Integrative and Comparative Biology* 42: 436-468.
21. Sick, T. J., Rosenthal, M., LaManna, J. C., and Lutz, P. L. 1982. Brain Potassium Ion Homeostasis, Anoxia, and Metabolic Inhibition in Turtles and Rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 243: 281-288.
22. Tsukahara, S., Kakeyama, M., and Toyofuku, Y. 2006. Sex Differences in the Level of Bcl-2 Family Proteins and Caspase-3 Activation in the Sexually Dimorphic Nuclei of the Preoptic Area in Postnatal Rats. *Journal of Neurobiology.* 66: 1411-1419.
23. Forger, N. G., Rosen, G. J., Waters, E. M., Jacob, D., Simerly, R. B., and de Vries, G. J. 2004. Deletion of Bax Eliminates Sex Differences in the Mouse Forebrain. *Proceedings of the National Academy of Sciences.* 14: 13666-13671.
24. Hara, A., Iwai, T., Niwa, M., Uematsu, T., Yoshimi, N., Tanaka, T., and Mori, H. 1996. Immunohistochemical Detection of Bax and Bcl-2 Proteins in Gerbil Hippocampus Following Transient Forebrain Ischemia. *Brain Research* 4: 249-253.

25. Zhou, J. G., Liu, J. C., and Fang, Y. Q. 2000. Effect of Hyperbaric Oxygen on the Expression of Proteins Bcl-2 and Bax in the Gerbil Hippocampus CA1 Following Forebrain Ischemia Reperfusion. *Zhongguo Ying Yong Sheng Li Xue Za Zhi.* 16: 298-301.
26. Mooney, S. M., and Miller, M. W. 2001. Effects of Prenatal Exposure to Ethanol on the Expression of Bcl-2, Bax and Caspase 3 in the Developing Rat Cerebral Cortex and Thalamus. *Brain Research* 17: 71-81.
27. Shindler, K. S., Yunker, A. M., Cahn, R., Zha, J., Korsmeyer, S. J., and Roth, K. A. 1998. Trophic Support Promotes Survival of Bcl-x-Deficient Telencephalic Cells *in vitro. Cell Death and Differentiation.* 5: 901-910.
28. Shin, C. M., Chung, Y. H., Kim, M. J., Shin, D. H., Kim, Y. S., Gurney, M. E., Lee, K. W., and Cha, C. I. 2000. Immunohistochemical Study on the Distribution of Bcl-2 and Bax in the Central Nervous System of the Transgenic Mice Expressing a Human Cu/Zn SOD Mutation. *Brain Research* 887: 309-315.
29. Wang, D. G., Johnston, C. F., Atkinson, A. B., Heaney, A. P., Mirakhur, M., and Buchanan, K. D. 1996. Expression of Bcl-2 Oncoprotein in Pituitary Tumours: Comparison with C-myc. *Journal of Clinical Pathology* 49: 795-797.
30. Turner, H. E., Nagy, Z., Gatter, K. C., Esiri, M. M., Wass, J. A., and Harris, A. L. 2000. Proliferation, Bcl-2 Expression and Angiogenesis in Pituitary Adenomas: Relationship to Tumour Behaviour. *British Journal of Cancer* 82: 1441-1445.
31. McCormick, C. A., and Hernandez D. V. 1996. Connections of Octaval and Lateral Line Nuclei of the Medulla in the Goldfish, Including the Cytoarchitecture of the Secondary Octaval Population in Goldfish and Catfish. *Brain, Behavior and Evolution* 47: 113-131.
32. Peter, R. E., Macey, M. J., and Gill, V. E. 1975. A Stereotaxic Atlas and Technique for Forebrain Nuclei of the Killifish, *Fundulus heteroclitus. Comparative Neurology* 159: 103-127.
33. Nieuwenhuys, R. 1963. The Comparative Anatomy of the Actinopterygian Forebrain. *Journal Hirnforsch* 6: 171-192.
34. Sheldon, R. E. 1912. The Olfactory Tracts and Centers in Teleosts. *Comparative Neurology* 22: 177-339.

ได้รับบทความวันที่ 12 มกราคม 2554
ยอมรับตีพิมพ์วันที่ 4 มีนาคม 2551